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A Summary of Vitamin D Assay Work in Maryland

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A SUMMARY OF VITAMIN D ASSAY WORK IN MARYLAND

As a result of the general recognition of the importance of an adequate supply of vitamin D in poultry rations, the market for anti-rachitic supplements has undergone great expansion during the past several years. The majority of commercial poultry mashes registered by the Maryland Feed Inspection Service now contain some ingredient included as a vitamin D supplement. Also large quantities of cod liver oil and other vitamin D carriers are retailed to livestock and poultry feeders who mix their own rations.

As early as 1930, Feed Control Officials appreciated the trend in the use of vitamin D supplements and foresaw the need for testing such products as a routine regulatory function.

At that time, the potency of vitamin D carriers was universally determined by means of the rat assay, a technic which had been highly developed. Most of the vitamin D supplements going into the feed industry were being used in poultry rations, however, and it was felt that a method of assay involving chick feeding would be more serviceable for feed control work. The demonstration that rats and chicks do not react in the same relative degree to vitamin D from various sources further amplified the advisability of a chick assay test for products designed for poultry feeding purposes.

As the result of experimental work in the Minnesota and Wisconsin feed control laboratories a procedure was developed which seemed promising. The method was tried out in several laboratories and found to give acceptable comparative results. With minor alterations the procedure was adopted as a tentative method by the Association of Official Agricultural Chemists in 1933.

The test, which became known as the A. O. A. C. method, is based on the percentage of ash produced in the tibiae of chicks fed for a four weeks period on a specific basal rachitic ration to which is added varying amounts of the product under assay. Chicks fed the basal ration alone become severely rachitic within four weeks, and show tibiae ash contents of from 30-34%. Chicks fed the basal ration supplemented with an adequate amount of vitamin D show tibiae ash contents of 44-48%. With insufficient vitamin D supplement the ash of the tibiae will fall in the range between 34% and 44% depending upon the severity of the deficiency.

A comparison of the potency of various oils is possible with the method by determining the smallest percentage of each which is just sufficient to produce a maximum ash content. In illustration, if it is found that the basal ration must be supplemented with ⅛% of oil A, ¼% of oil B, and ½% of oil C, in order to produce tibiae ash averages of 44% in chicks fed these supplemented rations, then oil A is approximately twice as potent in vitamin D as oil B, and oil B in turn is approximately twice as potent as oil C.

(A detailed description of the A. O. A. C. method as revised in 1936 is given on page 4 of this paper.)

In 1932, subsequent to a ruling by the Attorney General that products sold as vitamin carriers for poultry and livestock feeding were covered by the Maryland Feed Law, the Maryland Feed Inspection Service began the collection and assay of samples of vitamin D supplements being merchandised in Maryland. The procedure at first followed was to supplement the basal ration of the A. O. A. C. method with ⅛, ¼, and ½% of the respective products in the case of cod liver oils, and higher percentages of lower potency materials such as cod liver meal. The response in terms of bone ash production allowed a comparison of the potency of the various samples within the limits of the percentage ranges chosen. The results of these tests were published in Inspection Service bulletins.

Beginning January 1, 1933, all brands of vitamin D carriers were required to be registered with the Inspection Service, and certain labeling requirements were put into effect. The following year, in accord with recommendations of the Association of American Feed Control Officials, the Maryland Inspection Service

required also that the registration and label should carry a statement of guaranteed minimum potency by the A. O. A. C. method of assay. This potency was expressed as the percentage of the product which when added to the basal ration of the A. O. A. C. method would produce a minimum bone ash of 44%.

Samples were collected twice yearly when possible and tests made to determine whether the products were of the potency guaranteed. In reporting the test results, if the ash percentage found was 44% or more at the guaranteed level the product was reported as "Satisfactory." If a deficiency was found the test was reported as "Unsatisfactory."

This same procedure was followed in 1935 and 1936 except that the term "Guarantee Sustained" was substituted for "Satisfactory," and "Guarantee Not Sustained" was used in place of "Unsatisfactory" in reporting test results. Also in cases of deficiency the actual ash percentage obtained was shown.

During the years from the introduction of the chick test up to 1936 work was being conducted in several laboratories, notably in Minnesota and Wisconsin, in an effort to refine and improve the utility of the A. O. A. C. method. One of the most significant lines of study, involved the attempt to devise a means of expressing potency as evaluated by the A. O. A. C. method in terms of units of vitamin D. Facilitated by the availability of the accurately assayed U. S. Pharmacopoeia "Reference Oil," it was found possible to measure the response of chicks to rations carrying definite unitages of vitamin D. By comparing the bone ash of chicks fed rations of known vitamin D content from the reference oil with the ash percentages produced by definite percentage levels of test oils, it was found possible to calculate the potency of the latter in terms of units. The potency of the U. S. Pharmacopoeia "Reference Oil" is expressed in terms of "U. S. P. units" which are evaluated on the basis of a rigidly standardized rat test. Quite obviously, the results of the chick test could not be expressed as "U. S. P. units." An A. O. A. C. chick unit was thus devised and is described adequately in the following resolution adopted at the 1936 meeting of the Association of American Feed Control Officials: "Resolved that any material sold primarily as a vitamin D supplement for poultry shall be labeled with a minimum guarantee of *vitamin D potency expressed as Association of Official Agricultural Chemists' chick units of vitamin D per gram, determined according to the tentative procedure adopted by the Association of Official Agricultural Chemists and assuming that one U. S. P. unit of vitamin D from the U. S. Pharmacopoeia "Reference Oil" equals one Association of Agricultural Chemists' chick unit.*" The A. O. A. C. method was revised in 1936 so as to provide for evaluation in terms of units. At the same time the four weeks feeding period previously specified was reduced to a three-week period.

Following the resolution quoted above the Maryland Feed Inspection Service adopted the chick unit guarantee as registration and labeling requirements for 1937.

Immediately following is a detailed description of the recently revised A. O. A. C. method.

Vitamin D Assay By Preventive Biological Test

(Applicable to fish and fish liver oils and their extracts, and to materials used for supplementing the vitamin D content of feeds. Not applicable to irradiated ergosterol products or to irradiated yeast unless recommended for poultry.)

Basal Rachitic Ration

	Parts
Ground yellow corn	59
Pure wheat flour middlings	25
Crude domestic acid precipitated casein	12
Calcium carbonate (precipitated)	1
Calcium phosphate (precipitated)	1
Iodized salt (0.02% potassium iodide)	1
Non-irradiated yeast (7% minimum nitrogen)	1

Procedure

Place groups of 10 or more 1-day-old White Leghorn chickens in screen-bottomed biological cages or battery brooders out of direct sunlight. (Red electric light bulbs are satisfactory as a source of heat for the cages.) Reserve one group for negative control purposes, one or more groups for positive control purposes, and one or more additional groups for each material to be assayed. Keep distilled H_2O before the chicks at all times.

Prepare sufficient basal rachitic ration for the entire feeding period (60 pounds per 100 birds is ample). Prepare the supplemented rations at 8-12 day periods. Supplement the basal rachitic ration with corn oil in a quantity equal to the maximum addition of the oil to be assayed. (This is the ration to be fed to the negative control group.) Supplement the basal ration with one or more levels of an assayed cod liver oil, the level or levels to be so selected that the birds of one group will produce normal calcification. (These are the rations to be fed to the positive control group or groups.) Add corn oil to bring the percentage of oil up to that added to the negative control ration. (These are the rations to be fed to the other groups.)

On the second day give the groups two 15-minute feedings of their respective rations. Beginning the third day, feed the rations ad libitum for 21 days.

Kill the birds, remove the left tibia of each bird, and clear of adhering tissue. (To facilitate removal of adhering tissue, the bones may be placed in boiling H_2O for not more than two minutes.) If composite ashing of the tibiae of the groups is to be made, preserve the opposite tibiae in ethyl alcohol. Number the bones and place in 95% ethyl alcohol. Crush, wrap in filter paper, and extract the bones for 20 hours with hot 95% ethyl alcohol, followed by 20 hours with ethyl ether. (Other solvents may be used for this fat extraction.) Dry in a moisture oven, and store in a desiccator. Determine the percentage of ash of the moisture and fat-free bones by igniting individually or by group composite in a muffle furnace at approximately 850 degrees for one hour.

To obtain vitamin D potency of a product in terms of A. O. A. C. chick units, use the U. S. Pharmacopoeia "Reference Cod Liver Oil" in control rations at properly selected levels and compare the resulting control ash averages with the average produced by the material under assay. One A. O. A. C. chick unit of vitamin D is equal in biological activity for the chick to one unit of vitamin D in the U. S. P. Reference Cod Liver Oil in this method of assay.

A rather complete summary of the results of tests conducted on vitamin D supplements by the original four-week test, under the percentage guarantee in force during 1934-1936 is given in the table presented herein. Also included in the table are results of 1932 and 1933 tests which had been conducted at such levels that the results could be interpreted on the basis of the 1934 guarantees.

Following the table is an appendix which shows the form in which guarantees must be expressed for registration and labeling of vitamin supplements in Maryland. These forms correspond to those approved by other States carrying on enforcement work with vitamin D supplements.

Also in the appendix are quoted general recommendations, resolutions, and definitions of the Association of American Feed Control Officials, Inc., which are pertinent to the subject of vitamin supplements.

SUMMARY OF RESULTS OF TESTS

NOTE—No specific guarantees by the A. O. A. C. method were required for products sold in 1932 and 1933. A number of products registered under a guarantee in 1934 had been tested in 1932 and/or 1933 at levels at least as low as the 1934 guarantees, and are shown to add value to the table.

Manufacturer and Address	Name of Product	Guaranteed Percentage	Year of Test	Result of Test	
The Acton Company Philadelphia, Pennsylvania	Acton Pure Norwegian Cod Liver Oil, Not U. S. P.	½%	1934 Spring	Guarantee	Sustained
	Acton XX Pure Norwegian Cod Liver Oil Medicinal Grade, U. S. P.	½%	1934 Spring	"	"
			1935 Fall	"	"
	Acton Poultry Cod Liver Oil	½%	1935 Fall	"	"
	Acton Special Non-Freezing Cod Liver Oil	½%	1936 Spring	"	"
	Acton Norwegian Cod Liver Oil	½%	1936 Fall	"	"
Baugh & Sons Company Philadelphia, Pennsylvania	Norwegian Cod Liver Oil	½%	1936 Fall	Guarantee	Sustained
F. E. Booth Company, Inc. San Francisco, California	Crescent Brand Sardilene	¼%	1933 Fall	Guarantee	Sustained
			1934 Spring	Guarantee Not Sustained—40.2% Ash.	
			1935 Spring	Guarantee	Sustained
			1935 Fall	"	"
	Sardilene	½%	1936 Spring	"	"
			1936 Fall	"	"
California Packing Corporation San Francisco, California	Biotol (Sardine Oil)	⅜%	1935 Spring	Guarantee	Sustained
	Biotol, Biologically Tested Sardine Oil	⅜%	1935 Fall	"	"
			1936 Spring	"	"
			1936 Fall	"	"

Consumers' Import Company, Inc. New York City, New York	Cod Liver Oil	½%	1932 Fall	Guarantee	Sustained
	Cico Brand Medicinal Cod Liver Oil	½%	1933 Spring	"	"
	Cico Medicinal Cod Liver Oil	½%	1933 Fall	"	"
	Cico Cod Liver Oil	½%	1934 Fall	"	"
			1935 Spring	"	"
			1935 Fall	"	"
	Deluxe, U. S. P. Cod Liver Oil	¼%	1936 Spring	"	"
G. E. Conkey Company Cleveland, Ohio	Conkey's Y-O	2½%	1933 Fall	Guarantee	Sustained
			1935 Spring	"	"
			1936 Fall	"	"
Ezl. Dunwoody Company Philadelphia, Pennsylvania	Super X Cod Liver Oil	½%	1932 Fall	Guarantee	Sustained
			1935 Spring	"	"
			1935 Fall	Guarantee Not Sustained—38.07% Ash	
			1936 Spring	Guarantee	Sustained
			1936 Fall	"	"
	Super XX Cod Liver Oil, U. S. P.	½%	1933 Fall	"	"
	Super XX Medicinal Cod Liver Oil, U. S. P.	½%	1935 Spring	"	"
The Fox Company Newfield, New Jersey	Fox Pure Norwegian Cod Liver Oil, Medicinal	½%	1933 Spring	Guarantee	Sustained
	Fox Special Cod Liver Oil	½%	1933 Fall	"	"
			1934 Spring	"	"
			1935 Spring	"	"
			1935 Fall	"	"
	Fox Cod Liver Oil	½%	1936 Spring	Guarantee Not Sustained—42.37% Ash	
	Fox Quality Cod Liver Oil	½%	1936 Fall	Guarantee	Sustained

Gunning and Gunning New York City, New York	Gunning—Quality Cod Liver Oil	½%	1934 Spring	Guarantee	Sustained
			1935 Fall	"	"
			1936 Spring	"	"
			1936 Fall	"	"
Health Products Corporation Newark, New Jersey	Clo-Trate Concentrated Cod Liver Oil	⅛%	1932 Fall	Guarantee	Sustained
			1935 Spring	"	"
			1935 Fall	"	"
			1936 Spring	"	"
	Clo-Trate	⅛%	1936 Fall	"	"
Chas. L. Huisking & Company, Inc. New York City, New York	Vitamine Brand Pure Norwegian Cod Liver Oil	¼%	1932 Fall	Guarantee	Sustained
			1933 Spring	"	"
			1933 Fall	"	"
			1934 Spring	"	"
	Vitamine Brand Cod Liver Oil, U. S. P.	¼%	1935 Spring	Guarantee Not Sustained—37.1% Ash	
			1935 Fall	Guarantee	Sustained
	Vitamine Brand Cod Liver Oil	¼%	1936 Spring	"	"
			1936 Fall	"	"
	High Hatchability Special Vitamine Brand Cod Liver Oil Reinforced with Vitamin E	¼%	1936 Spring	Guarantee	Sustained
King Brothers & Company Philadelphia, Pennsylvania	Kings Xtra Cod Liver Oil	½%	1935 Spring	Guarantee	Sustained
			1936 Fall	"	"
	Kings Poultry Cod Liver Oil	½%	1936 Spring	"	"
McKesson & Robbins, Inc. Bridgeport, Connecticut	Isdahl's Superfine Poultry Cod Liver Oil	½%	1933 Spring	Guarantee	Sustained
	Isdahl's Superior Poultry Cod Liver Oil	½%	1933 Fall	"	"
	Isdahl's Norweigian Cod Liver Oil	½%	1934 Spring	"	"
			1935 Spring	"	"
			1935 Fall	"	"
	Isdahl's Superfine Poultry U. S. P. Non-destearinated Cod Liver Oil	½%	1936 Spring	Guarantee	Sustained
	Midnight Sun Cod Liver Oil	½%	1936 Fall	Guarantee Not Sustained—35.56% Ash	

	Midnight Sun Cod Liver Oil	½%	1936 Fall	Guarantee Not Sustained—35.56% Ash	

Mutual Rendering Company, Inc. Philadelphia Pennsylvania	Mureco Cod Liver Oil	1%	1936 Spring	Guarantee	Sustained
National Oil Products Company Harrison, New Jersey	Nopco DD Cod Liver Oil	¼%	1933 Fall	Guarantee	Sustained
			1935 Spring	"	"
		⅜%	1935 Fall	"	"
			1936 Spring	"	"
	Nopco EE Cod Liver Oil	¼%	1934 Spring	"	"
	Nopco XX Vitamin Concentrate Added to Pure Cod Liver Oil	1/16%	1935 Spring	"	"
		3/32%	1935 Fall	"	"
			1936 Spring	"	"
	Nopco XX	3/32%	1936 Fall	"	"
	Nopco Non-Destearinated Cod Liver Oil	⅜%	1936 Fall	"	"
Pratt Food Company Hammond, Indiana	Pratt's Cod Liver Oil, Not U. S. P.	½%	1932 Fall	Guarantee	Sustained
			1933 Spring	"	"
	Pratt's Cod Liver Oil	½%	1933 Fall	"	"
			1934 Spring	Guarantee Not Sustained—41.6% Ash	
			1935 Spring	Guarantee	Sustained
			1935 Fall	"	"
			1936 Spring	"	"
			1936 Fall	"	"
Robert A. Reichard, Inc. Allentown, Pennsylvania	Keystone Brand U. S. P. Poultry Cod Liver Oil	½%	1936 Fall	Guarantee	Sustained
Sea Board Supply Company Philadelphia, Pennsylvania	Cod Liver Oil	½%	1936 Fall	Guarantee	Sustained

Silmo Chemical Company Vineland, New Jersey	Silmo XX Cod Liver Oil	⅛%	1933 Spring	Guarantee	Sustained
			1934 Spring	"	"
			1935 Spring	"	"
			1935 Fall	Guarantee Not Sustained—43.3% Ash	
			1936 Fall	Guarantee	Sustained
	Silmo Poultry D Cod Liver Oil	1%	1933 Fall	"	"
		½%	1934 Spring	"	"
		1%	1935 Spring	"	"
			1935 Fall	"	"
			1936 Fall	"	"
Whitmoyer Laboratories, Inc. Myerstown, Pennsylvania	Whitmoyer Quality Cod Liver Oil	½%	1936 Fall	Guarantee	Sustained

Appendix

1. The following information must be shown on each container of vitamin D supplements merchandised in the State of Maryland.

   (a) Net weight or quantity;

   (b) Name of product;

   (c) Vitamin D Potency: Guaranteed to contain not less than (?) Association of Official Agricultural Chemists' chick units of vitamin D per gram:

   (d) Name and address of manufacturer or party responsible for sale.

2. The following resolution has been adopted by the Association of American Feed Control Officials, Inc.

   Resolved that any material sold primarily as a vitamin D supplement for poultry shall be labeled with a minimum guarantee of vitamin D potency expressed as Association of Official Agricultural Chemists' chick units of vitamin D per gram, determined according to the tentative procedure adopted by the Association of Official Agricultural Chemists, and assuming that one U. S. P. unit of vitamin D in the U. S. Pharmacopoeia "Reference Cod Liver Oil" equals one Association of Official Agricultural Chemists' chick unit.

3. The following definitions have been adopted as official definitions by the Association of American Feed Control Officials, Inc.

   Cod Liver Oil is the product obtained by extraction of part of the oil from cod livers.

   Sardine Oil or Pilchard Oil is the product obtained by extraction of part of the oil from the whole Pacific sardine or pilchard or from cannery refuse of this species of fish.

   Salmon Oil is the product obtained by extraction of part of the oil from the cannery refuse of salmon.

   Tuna Oil is the product obtained by extraction of part of the oil from the cannery refuse of tuna.

   Menhaden Oil is the product obtained by extraction of part of the oil from whole menhaden.

   Herring Oil is the product obtained by extraction of part of the oil from the whole herring or part of the herring.

   Salmon Liver Oil is the product obtained by extraction of part of the oil from salmon livers.

   Stearine is the solid material obtained by filtration from an animal or vegetable oil after chilling or freezing in the process of refining. When this product is sold as a feed, the term shall be prefixed by the name of the oil from which it is obtained.

4. The following are tentative definitions of the Association of American Feed Control Officials, Inc.

   Irradiated Yeast is yeast which has been irradiated in order to increase its anti-rachitic potency and shall contain at least 45 per cent protein on the moisture free basis. (NOTE—If yeast or irradiated yeast consists of bakers' type yeast, the term "bakers' type" shall precede the word yeast; if it consists of brewers' type yeast the term "brewers' type" shall precede the word yeast.)

Cod Liver Oil is the oil obtained from the livers of gadus morrhuae and/or other species of the family gadidae. It must contain not less than 600 U. S. P. units of vitamin A and not less than 85 Association of Official Agricultural Chemists' chick units of vitamin D per gram.

Fortified Cod Liver Oil is cod liver oil mixed with other suitable fish oils and/or fish oil concentrates so as to contain not less than 400 Association of Official Agricultural Chemists' chick units of vitamin D per gram and shall be labeled with the minimum guarantee of vitamin A potency in terms of U. S. P. units of vitamin A per gram.

NOTE—Tentative definitions have received consideration, but are not official. They are subject to revision before final adoption.